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CHEMICAL ANALYSIS OF THE MALE AEDEAGAL BLADDER IN THE FIRE ANT, *SOLENOPSIS INVICTA* BUREN[1]

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Abstract.—The aedeagal bladder in the male reproductive system of the fire ant *Solenopsis invicta* Buren lies within the external genitalia. The lumen is lined by a single layer of squamous epithelium surrounded by heavy musculature. In mature, sterile and mated males it contains a milky substance but no fluid is found in newly eclosed males. The fluid in the bladder consists of fatty acid esters (glycerol monodecanoate and glycerol monododecanoate) in a proteinaceous matrix. The function of the secretion remains unknown.

The male reproductive system in the imported fire ant, *Solenopsis invicta* Buren consists of the testes, vas deferens, seminal vesicles, accessory gland, ejaculatory duct, wedge, and external genitalia (Ball and Vinson, 1984). An additional organ, the aedeagal bladder, has been described in many species of male ants but its function is unknown and no chemical analyses of its contents have been reported.

The aedeagal bladder was first described by Clausen (1938). Forbes (1954) discussed the anatomy and histology of the aedeagal bladder in *Camponotus pennsylvanica* DeGeer in considerable detail. Forbes (1958) also described the aedeagal bladder in the army ant, *Eciton hamatum* F. Later, Forbes and Do-Van-Quy (1965) investigated the histology of the bladder in the legionary ant, *Neivamyrmex harrisi* (Haldeman). Ford and Forbes (1980) showed that the bladder is present in several species of doryline ants and Hagopian (1963) has described an aedeagal bladder in the ponerine ant, *Rhytidoponera metallica* F. Smith. In each case, the aedeagal bladder was found to be an ovoid muscular sac lined with squamous epithelium, filled with a basophilic granular mass and emptying posteriorly into the region of the ejaculatory duct between the inner genitalic valves.

Trakimas (1967) described an aedeagal bladder in *Myrmica rubra* L. but she stated that the granular material in the lumen stains acidophilicly and is found in a greater amount in mature males with degenerated testes. Tice (1967) did an extensive study on the male reproductive system of the black imported fire ant, *Solenopsis saevissima richteri* Forel, and also reported a muscular aedeagal bladder with a granular acidophilic substance in the lumen. Ball and Vinson (1984) found an aedeagal bladder lined with a single layer of squamous epithelium in the male reproductive system of the red imported fire ant, *S. invicta* Buren, and determined that the material in the lumen contains a small amount of protein. They also determined that the bladder material is found in approximately equal amounts in mature, mated and sterile males.

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The purpose of this investigation was to determine the chemical composition of the contents of the aedeagal bladder in the fire ant, *S. invicta.*

MATERIALS AND METHODS

Dissection and collection procedures. Newly eclosed (one day old) males and sterile males (Hung et al., 1974) at least two weeks old were collected from laboratory colonies. Sterile males lack testes and are, therefore, incapable of sperm production. Additional males, collected as they flew from the surface of the mound prior to mating (preflight) and after they returned (postflight) were also examined.

Males were pinned through the ventral surface of the thorax, the terminal gastral segments were grasped with microforceps and pulled apart exposing the external genitalia, the claspers were carefully pulled and the accessory glands were severed from the claspers. The claspers were then quickly positioned with the posterior end of the genital valves pointing upward and the muscular contractions within the claspers caused the expulsion of the aedeagal bladder material which was collected by capillary action using a flame-drawn pipette.

Chemical analysis. A variety of chemicals was used to test the solubility of the aedeagal bladder material. Samples were collected from 5 mature males and placed into sterilized glass tubes containing either acetone, ethanol, pentane, 0.1 N NaOH, 0.1 N HCL or distilled water (pH 7).

During dissection of the aedeagal bladder, the expelled material appeared as a white particulate material resembling deposits of uric acid. Therefore, the material was collected from 20 mature males, placed into 0.1 N NaOH and tested concurrently with a standard uric acid sample on thin layer chromatography (TLC) on cellulose using 0.1 N NaOH as the solvent. The plate was then examined under ultraviolet light. The material also appeared to have droplets of suspended oil indicating lipid-like substances. To determine the presence of free lipids, the aedeagal bladder material was collected from 40 males and placed into ethyl ether. Standard samples (Sigma Chem. Corp., St. Louis, Mo. 63178) of glycerides (distearin, triolein, and monopalmitin), a fatty acid (oleic acid), a steroid (stigmasterol) and an ester (palmitic acid ethyl ester) were mixed with chloroform to obtain final concentrations of 1 μg/μl. Five and 10 μl drops of the sample and standards were spotted onto a silica gel TLC plate and air dried. The plate was then placed in a chromatography tank with a hexane:ethyl ether solvent (50:50). The solvent front was allowed to migrate to within 10 mm of the top and the plate was then transferred to an iodine developing tank. Aedeagal bladder material and standard spots were then compared and RF values computed.

As a further analysis, the material, collected from 40 mature males, was dissolved in pentane as previously determined and injected onto a column gas chromatograph (GC). The samples were analyzed on a Tracor 550 GC with a flame ionization detector modified with an all glass variable split ratio collection system. Separations were obtained on a 1.83 M × 4 mm i.d. column of 3% OV-101 on Chromosorb 750, 100–120 mesh at a nitrogen flow rate of 60 ml/min programmed from 50 to 270°C at 10°C/min. Standard monoglycerides were analyzed under the same conditions. Mass spectral (MS) analyses were performed on a Finnigan 1020 OWA quadrupole GC-

Table 1. RF values for TLC lipid analyses of standard and gland extract samples. Solvent—ethyl ether/hexane (50:50), iodine vapor visualization on silica gel F254 plate.

Sample	RF value
Monoplatmitin	0.05
Distearin	0.39
Triolein	0.61
Oleic acid	0.45
Stigmasterol	0.67
Palmitic acid ethyl ester	0.67
Gland extract	0.05

MS unit using a 12 M × 0.33 mm i.d. BPI column, 6 psi, temperature programmed from 60 to 220°C at 20°C/min. Monoglyceride standards (Sigma Chem. Corp., St. Louis, Mo. 63178) were used, with RT 9.41 min for C_{10} and 10.65 min for C_{12} under the above conditions. Hexane gland extracts were subjected to GC-MS analysis without preliminary purification.

The polyacrylamide disc gel electrophoresis procedure described by Davis (1964) was used for verification of the presence of lipoproteins. A 7.5% separating gel was allowed to polymerize in glass tubes (i.d. 4.5 mm, length 75 mm) precleaned in chromic acid followed by the addition of a 3.3% stacking gel to the top of the separating gel. The aedeagal bladder material from 20 males was collected and placed in 100 μl of an aqueous 1% mercaptoethanol/1% SDS disruption buffer solution. Standards (Sigma Chem. Corp., St. Louis, Mo. 63178) consisting of 100 μg each of phosphorylase A (330 K), IgG (150 K), bovine serum albumin (67 K), ovalbumen (43.5 K) and cytochrome C (12.5 K) were also placed in 100 μg of disruption buffer. Standards and the aedeagal bladder sample were heated for 15 min at 60°C before applying 30 μl aliquots per tube. Electrophoresis proceeded at 8 mA per gel tube for ca. 4 hr.

A Cahn gram electrobalance was used to determine the percent composition of proteins and/or lipids in the aedeagal bladder material. The contents of the bladder from 30 mature males were extracted, placed on aluminum foil pans, weighed and moved to a desiccator. Once a constant weight was obtained, the material was immersed 3 times in hexane and returned to the desiccator. After reaching a constant weight again, the material was placed 3 times in chloroform : methanol (50:50) for 5 min each. Two samples and a control (empty pan) were analyzed concurrently.

Transmission electron microscopy was used to observe the ultrastructure of the bladder material. The claspers with the intact aedeagal bladder were removed from several mature males and fixed in 2% glutaraldehyde/2% acrolein/1.5% DMSO in 0.133 M sodium cacodylate buffer (pH 7.4) at room temperature for 6 hr. The specimens were then rinsed 3 times in buffer and post-fixed in 2% osmium tetroxide in 0.1 M buffer and 0.2 M sucrose for 3 hr. The samples were rinsed 3 times in 0.1 M buffer, 2 times in distilled water and transferred to 1% aqueous uranyl acetate overnight. After dehydration in an ethanol series, the specimens were embedded in

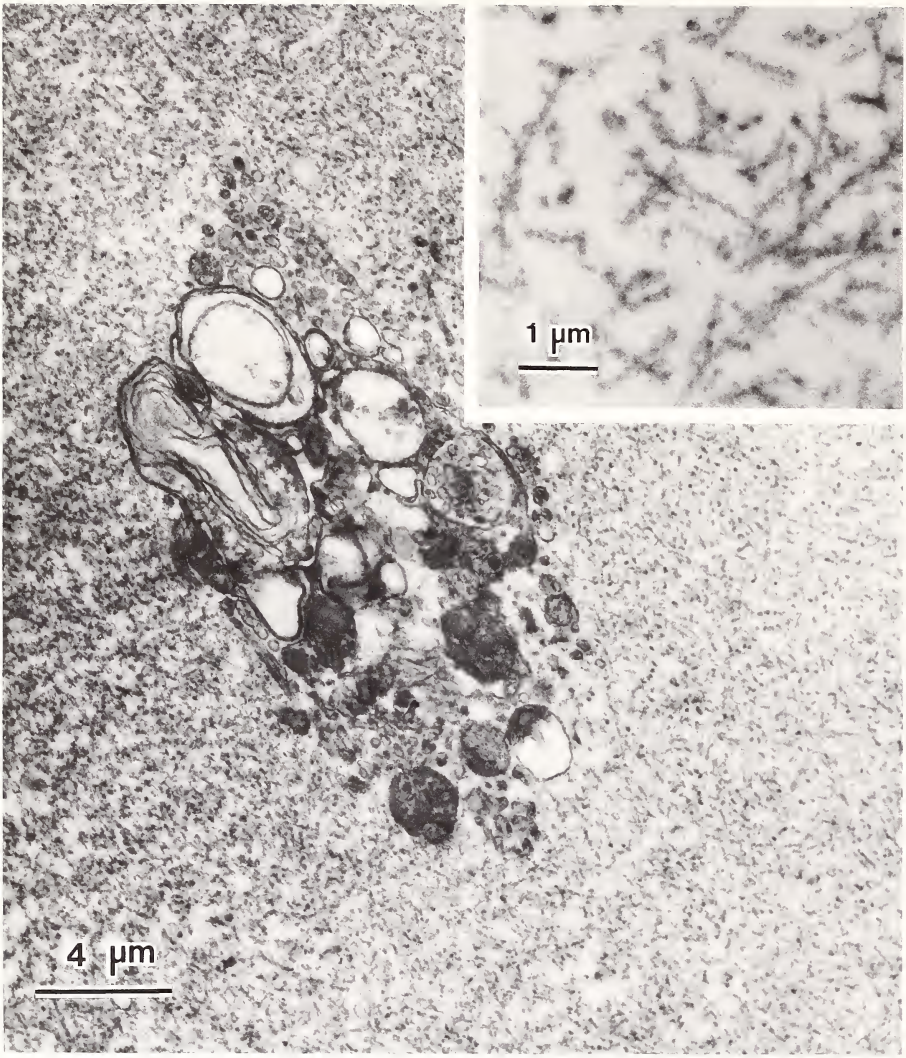

Fig. 1. Transmission electron micrograph of the aedeagal bladder material surrounding a lysed cell. Insert: Magnification of the fibrillar material.

Epon araldite resin, vacuum aspirated and allowed to polymerize for 24 hr at 40°C and then 48 hr at 60°C. The blocks were sectioned at 500 Å, stained with Toluidine blue and viewed on a Ziess 10C/CR transmission electron microscope at 80 kV.

RESULTS AND DISCUSSION

Chemical analysis. Upon dissection in distilled water, the aedeagal bladder material appeared as a white, granular substance and was insoluble in water, although a few

suspended oil droplets were observed. Only slight dissolution occurred when the extract was placed in acetone and ethanol. However, the material completely dissolved in 0.1 N NaOH but not in 0.1 N HCL.

TLC analysis for uric acid was negative for the gland extract. Additional TLC analysis for the presence of free lipids showed that the material contained monoglycerides as shown by equal RF values (Table 1).

GC analysis of several samples showed gland contents to be variable perhaps due to contamination during dissection but most samples (4 out of 6) showed the presence of peaks with retention times equal to those of monodecanoyl and/or monododecanoyl glycerol. These samples were subjected to GC-MS analysis. Materials with retention times equal to those of the monoglyceride standards had mass spectra identical to those of the standards. Monododecanoyl glycerol was present in 4 of the 6 samples; monodecanoyl glycerol was in 1 sample in a 1:10 ratio. Other unidentified highly variable materials made up from 10 to 80% of the samples containing monoglycerides. Monoglyceride spectra were in excellent agreement with those given in the EPA/NIH Mass Spectral Data Base (Heller and Milne, 1978).

Polyacrylamide gel electrophoresis showed the separation of one large band with a molecular weight of ca. 40–90 K indicating the presence of lipoprotein(s).

The results using the gram electrobalance showed that nearly all of the aedeagal bladder material was composed of polar lipids since the material was insoluble in hexane but completely dissolved in chloroform : methanol.

Transmission electron microscopy showed the presence of several lysed cells surrounded by slender fibrillar material in the bladder substance (Fig. 1). Closer examination revealed miniscule branching with dark, round globules adhering to the surface of the fibrils (Fig. 1, insert).

This study has shown that the aedeagal bladder in the male fire ant, *S. invicta,* consisted of two monoglycerides in a protein matrix. The source of this material is unknown, and does not appear in the bladder until 5 to 7 days post emergence. No change in the epithelial cells of the aedeagal bladder in preflight or postflight males was observed nor did the cells appear to be secretory. The function of the gland also remains unknown. Since the aedeagal bladder material has been found in approximately equal amounts in preflight and mated, postflight males, it seems unlikely that it is used primarily during mating.

Several studies have shown that lipids play a major role in the biology of the fire ant. Vinson et al. (1967) demonstrated that the triglyceride of linoleic acid was the most active phagostimulant for workers. Vander Meer et al. (1982) analyzed several glands in mated queens of various ages and found large amounts of triacylglycerols in the crop and oesophagus which declined rapidly until 25 days following mating coinciding with the first brood production. Nelson et al. (1980) reanalyzed the cuticular methylalkanes in the cuticle of *S. invicta* and *S. richteri* Forel and determined that the triglycerides originally found by Lok et al. (1975) had different structures than were reported, although no function was demonstrated. However, the fatty acids listed in these reports were not the same as we found in the aedeagal bladder material.

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